Effects of Progesterone and Human Chorionic Gonadotrophin Administration Five Days Postinsemination on Plasma and Milk Concentrations of Progesterone and Pregnancy Rates of Normal and Repeat Breeder Dairy Cows

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ABSTRACT

Treatment with a progesterone-releasing intravaginal device between days 5 and 12 after estrus elevated ($p < 0.05$) plasma progesterone concentrations between days 6 and 8 in comparison with controls. Treatment with injectable progesterone (200 mg) on days 5, 7, 9 and 11 postestrus did not increase plasma progesterone concentrations over controls. The administration of 1500 IU human chorionic gonadotrophin (hCG) on day 5 after estrus resulted in a sustained increase ($p < 0.01$) in plasma progesterone concentrations from day 8 until day 20 when measurements ceased. Pregnancy rates, as a result of artificial insemination (AI) at the pretreatment estrus, in these treatments (n = 12-14 each), were unaffected by any of the treatments and ranged from 57.1 to 75.0% at 45-60 days post-AI. In a field trial, of 36 repeat breeder cows treated with 1500 IU hCG 5.5 days after insemination, 47.2% were pregnant at 60 days, whereas 39.5% of saline-treated controls were diagnosed pregnant. Treatment with hCG significantly ($p < 0.05$) increased milk progesterone concentrations over controls on days 14 and 20 after insemination.

RÉSUMÉ

Un traitement de progestérone, administré à l'aide d'un système de relâche intra-vaginal entre les jours 5 et 12 post-saillie, a augmenté ($p < 0,05$) les concentrations plasmatiques de progestérone entre les jours 6 et 8 par rapport aux animaux témoins. L'injection de progestérone (200 mg) aux jours, 5, 7 9 et 11 post-oestrus n'a pas augmenté les concentrations plasmatiques de progestérone par rapport aux témoins. L'administration de 1500 UI de l'hormone chorionique gonadotrope humaine (HCG) le jour 5 post-oestrus à resulté en une augmentation soutenue des concentrations plasmatiques de progestérone du jour 8 au jour 20 alors que les prélèvements ont été inter-rompus. Les taux de gestation, après insémination artificielle (IA) suivie des traitements (n = 12-14 vaches/traitement), n'étaient pas améliorés et variaient de 57,1 à 75% aux jours 45-60 post-IA. Suite à un essai clinique utilisant 79 vaches avec saillies repétées dont 36 ont été traitées avec 1500 IU d'HCG 5,5 jours post-IA, 47,2% (n = 17) étaient gravides à 60 jours alors que 39,5% (n = 17) des témoins (n = 43) traitées avec la saline étaient diagostiquées gravides. Le traitement avec la HCG a augmenté significativement ($p < 0,05$) les concentrations de progestérone dans le lait par rapport aux témoins les jours 14 et 20 post-IA.
(Traduit par Dr Denis Vaillancourt)

INTRODUCTION

It is often considered that early embryonic mortality is responsible for a substantial proportion of pregnancy failures, especially in cows which have already received a number of inseminations (1,2). It is common for therapeutic treatment to involve the administration of progesterone in an attempt to assist in the support of the early stages of pregnancy (3,4). For example, we have previously demonstrated that progesterone-releasing intravaginal devices (PRID) were effective in increasing circulating progesterone concentrations and pregnancy rates when placed in the vagina of dairy cows between days 5 and 12 or days 12 through 17 post-insemination (5). At the same time, it was also found that supplementation with progesterone was often negated by a tendency of the animals under treatment to reduce progesterone synthesis, especially later in the cycle. Given that PRIDs are unavailable to dairy farmers in North America we decided to investigate alternative treatments to increase circulating progesterone concentrations and improve the opportunity for cows to remain pregnant.

Initially, a preliminary experiment was designed to compare alternate methods for elevating plasma progesterone concentrations using injected progesterone, human chorionic gonadotrophin (hCG), and PRID in comparison with untreated controls. On the basis of these results, a field trial on the effectiveness of hCG treatment in raising milk progesterone concentrations and pregnancy rates was conducted.

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MATERIALS AND METHODS

PRELIMINARY EXPERIMENT

Lactating Holstein cows at the Elora Dairy Research Centre, University of Guelph, were randomly allocated to treatment after the first artificial insemination (AI) following the voluntary waiting period (60 to 80 days postpartum). Fourteen control animals received no treatment, 13 animals had a PRID (CEVA Laboratories, Kansas, estradiol capsule removed) inserted on day 5 post-AI followed by removal on day 12. Twelve cows were treated with progesterone by injection (Centra Progestin, Central Sales Ltd., Brampton, Ontario) at a dose of 200 mg/animal on days 5, 7, 9 and 11 postinsemination. Fourteen cows were treated with 1,500 IU hCG (Progon, PVU Inc., Victoriaville, Quebec) on day 5 postinsemination. Cows were inseminated using semen from a large battery of proven Holstein sires which were randomly distributed across all treatments. For all cows, starting four days postinsemination, blood samples were removed from the coccygeal vein/artery at daily intervals until 21 days after insemination. To ensure cows were inseminated at the correct time, continuing milk samples were taken and assayed for progesterone (5) from estrus onwards and only those cows that were inseminated with base-line progesterone (< 0.2 ng/mL) were included in the analysis. Cows were observed for return to service and were palpated rectally to determine pregnancy status 45 to 60 days postinsemination. Milk progesterone determinations were continued during this period to identify the incidence of embryonic mortality and also as a check on estrous detection and pregnancy determination. Both milk and plasma progesterone concentrations were determined using previously validated radioimmunoassays (5) and the results collated over time.

FIELD TRIAL

The field trial was designed as a randomized, blind experiment. Holstein cows from farms serviced by the Ontario Veterinary College Ruminant Health Management Clinic that had been bred three or more times and were free of clinical ovarian or uterine pathology were entered into this experiment. A total of 82 repeat-breeder cows from 24 farms were ultimately included. The cows were of a mean age of 5.5 yr and were a mean of 150 days from parturition to the treatment service. Treatments were administered between four and six days postinsemination such that the mean interval from service to treatment was 5.5 days. Cows within farms were randomly assigned as pairs to treatment intramuscularly with either 1,500 IU hCG extended to 5 mL with 0.9% saline or 5 mL 0.9% saline alone. At the time of treatment, a milk sample was taken and preserved with potassium dichromate for subsequent progesterone assay by radioimmunoassay. Milk samples were also taken 14 and 20 days postinsemination for radioimmunoassay of progesterone and pregnancy status was confirmed at rectal palpation by experienced veterinarians 45 to 60 days postbreeding.

STATISTICAL ANALYSES

Comparisons between treatments for pregnancy rates were determined using Fisher's exact test or Chi-square analysis. Comparisons among means were conducted largely using Student's *t*-tests, using the Bonferroni adjustment for unequal variances as necessary. All statistical procedures were conducted using PC-SAS.

RESULTS

PRELIMINARY EXPERIMENT

Pregnancy rates did not differ across treatments and ranged from 57 to 75% (Table I). Plasma progesterone concentrations in the various treatment groups are described in Fig. 1. Progesterone concentrations were increased ($p < 0.05$) by the administration of PRID, but only between days 5 and 8 postestrus. Cows treated with injectable progesterone had similar ($p > 0.05$) progesterone concentrations to control cows throughout. It should be noted that blood samples were taken immediately prior to treatment. Presumably, the progesterone from the injection was rapidly excreted such that the progesterone level was not elevated when the sample was taken the next day. The only treatment that provoked a sustained increase in progesterone over controls was hCG. This difference ($p < 0.01$) was not evident until day 8 postestrus but was sustained thereafter. Rectal palpation of the ovaries of these animals on days 14 through 16 of the treatment cycle revealed the presence of one or two supranumary corpora lutea on the ovaries. Across all treatments, cows which did not become pregnant returned to estrus within 21 ± 3 days of their previous estrus and became pregnant to subsequent inseminations.

FIELD TRIAL

There were no differences in age (5.6 versus 5.3 yr) or service number at the time of treatment (4.3 versus 4.0) for control versus hCG-treated cows, respectively. The pregnancy rates and milk progesterone concentrations for these animals are given in Table II. Pregnancy rate was not different (47.2% versus 39.5%) between hCG-treated and control cows. Treatment with hCG did, however, significantly increase milk progesterone concentrations on both days 14 and 20 postbreeding.

Since it is important that the hCG treatment should not interfere with the normal return to estrus in cows which do not become pregnant, the milk progesterone concentrations for the day 20 samples were divided both into treatment and pregnancy status and

TABLE I. Effect of progesterone and human chorionic gonadotrophin (hCG) treatment on pregnancy rates in Holstein cows

Treatment	Control	PRID[a]	Progesterone[b]	hCG[c]
No. of cows	14	13	12	14
No. pregnant	8	8	9	8
% pregnant	57.1	61.5	75.0	57.1

[a]PRID — Progesterone-releasing intravaginal device from days 5-12 post-AI
[b]Progesterone — 200 mg Centra Progestin on days 5, 7, 9 and 11 post-AI
[c]hCG — 1500 IU of human chorionic gonadotrophin on day 5 post-AI

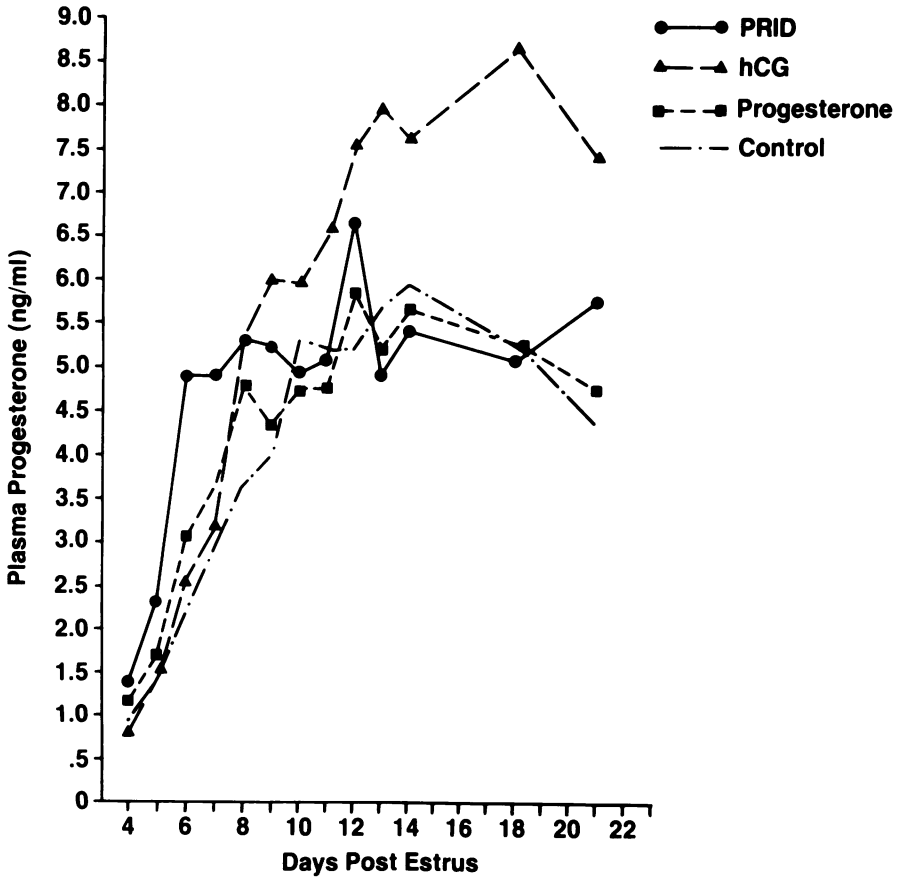

Fig. 1. Effect of treatment with 1) PRID (progesterone-releasing intravaginal device) between days 5-12 postestrus; 2) 200 mg progesterone on days 5, 7, 9 and 11 postestrus; 3) 1500 IU human chorionic gonadotrophin (hCG) on plasma concentrations of progesterone in inseminated Holstein cows. Data from untreated controls are included for comparison; n = 12-14 across treatments. Means are different (at least $p < 0.05$) from controls for PRID, days 6, 7, 8; hCG days 8-21. Pregnancy rates are presented in Table I.

these results are illustrated in Table III. It can be observed that in nonpregnant cows, progesterone concentrations were not different between saline and hCG-treated groups. It can also be observed that the effect of hCG on progesterone concentrations persisted until day 20 in those animals which became pregnant.

DISCUSSION

The objective of this research was to develop methods for increasing concentrations of progesterone between days 5 and 20 postinsemination. This was considered an appropriate strategy, in that it has previously been demonstrated that pregnant heifers have higher concentrations of progesterone than nonpregnant heifers at this time (6,7), and that a quiescent, progesterone-dominated uterus is essential for the establishment of the conceptus within the uterine lumen (8,9).

As in our previous studies (5), PRID was effective in supplementing progesterone concentrations, but only for the first three days after its insertion. This agrees with previous studies which demonstrated that the effect of PRID in supplementing progesterone is at least partially abrogated by a reduction in endogenous luteal progesterone output (5). Similarly, a sustained elevation of progesterone concentrations could not be demonstrated following treatment with an injectable preparation, even though this product was supposedly long-acting.

In terms of elevating progesterone concentrations for most of the period in question, the only successful treatment was the administration of hCG on day 5. This agrees with a previous study where hCG was administered early in the estrous cycle (10). It should be noted that the effect of hCG treatment was not apparent until day 8 postestrus. This, together with the palpation data, indicated that these animals formed additional corpora lutea after treatment. It has recently been demonstrated that the injection of hCG causes luteinization of large follicles which are present at the time of treatment (11). Since it takes two to three days for the resulting corpora lutea to generate substantial amounts of progesterone, this would account for the delay in the increase in progesterone concentrations following treatment (12). In a separate study, four beef heifers were treated in an identical fashion and their ovaries were recovered at slaughter on day 12 of the estrous cycle. Inspection of the ovaries revealed that each of these animals had two corpora lutea of differing age, one a 12 day corpus luteum from the original estrus, the second an induced corpus luteum approximately four days of age (13), confirming the observations of Price and Webb (11).

Despite the considerably elevated progesterone concentration in the hCG-treated animals, however, there was no effect of treatment with either

TABLE II. Milk progesterone and pregnancy results in repeat breeder cows treated with hCG (1500 IU hCG on day 5 post-AI) or saline control

	Saline	hCG treated
No. of cows	43	36
No. pregnant (%)	17 (39.5)[a]	17 (47.2)[a]
Milk progesterone on:		
Day 5 (pretreatment) (ng/mL)	0.9 ± 0.10[a]	1.4 ± 0.31[a]
Day 14 (ng/mL)	3.2 ± 0.30[a]	4.3 ± 0.46[b]
Day 20 (ng/mL)	2.9 ± 0.30[a]	4.1 ± 0.60[b]

[a,b]Means are different $p < 0.05$

TABLE III. Milk progesterone concentrations on day 20 after AI in repeat breeder cows in relation to treatment and pregnancy status

Treatment	No. of cows	Pregnancy status	P_4 (ng/mL) ± SEM
Saline	26	Not pregnant	3.0 ± 0.55[a]
Saline	17	Pregnant	2.7 ± 0.45[a]
1500 IU hCG	19	Not pregnant	2.5 ± 0.65[a]
1500 IU hCG	17	Pregnant	5.8 ± 0.86[b]

[a,b]Means are different $p < 0.05$

hCG or, indeed, any of the other treatments on pregnancy rates. Any cows that did not become pregnant after hCG treatment returned to estrus at approximately the normal time, such that the treatment did not interfere with normal rebreeding.

It should be noted that the inherent fertility of this group of animals was good. In our previous study (5), the effect of progesterone on improving pregnancy rates was observed in a situation where fertility was relatively poor. With this in mind, it was decided that hCG treatment on day 5 was an appropriate technique for stimulating increased progesterone concentrations during this critical phase of early pregnancy and was worthy of testing in a larger experiment using cows with compromised fertility, to determine whether it would be effective in improving pregnancy rates under field conditions.

Cows in the field trial were selected on the basis of a history of unsuccessful artificial inseminations, and were regarded as subfertile cows. Pregnancy rates in the experiment were, therefore, somewhat better than expected, with 39.5% of controls and 47.2% of treated animals becoming pregnant to the treatment insemination. This may indicate that the animals selected for treatment had not become pregnant because of inadequate management practices, which were, to a certain extent, remedied by a response of producers to the implementation of an experimental protocol. However, since neither the producers nor the clinicians were aware of which cows received which treatment, this would not represent a bias in the experimental procedure.

Under these conditions, as in (10), there was no effect of hCG on pregnancy rate, although hCG treatment elevated progesterone concentrations in milk samples taken on day 14. This effect was sustained through day 20, such that cows which became pregnant following treatment with hCG had elevated progesterone concentrations relative to cows which were not pregnant or treated with saline. These data indicate the prolonged effect of treatment, presumably because the accessory corpora lutea (11) were sustained for a relatively long period of time. Unfortunately, these cows were not studied any further and we do not know at this time whether the accessory corpora lutea lasted the entire gestation. It was also interesting that milk progesterone concentrations were higher than expected 21 days after insemination in those cows subsequently diagnosed as not pregnant.

In conclusion, therefore, treatment with hCG on day 5 after insemination will substantially elevate progesterone concentrations during at least the next two weeks. In cows which do not become pregnant, this treatment does not interfere with the normal return to estrus and it is, therefore, an effective way of creating supplemental progesterone concentrations in dairy cows. While the current experiments did not indicate any positive effect of this treatment on pregnancy rates, there were also no contraindications. In situations, therefore, where it may be suspected that inadequate progesterone concentrations may be involved in poor fertility of dairy cattle, this treatment could be regarded as being superior to supplementation with exogenous progesterone since continuously elevated progesterone concentrations result from a single treatment.

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